Menopausal Hormone Therapy and Risk of Neuropsychiatric Disease: A Drug Target Mendelian Randomisation Study

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Menopausal Hormone Therapy and Risk of Neuropsychiatric Disease: A Drug Target Mendelian Randomisation Study

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Abstract

The effects of menopausal hormone therapy (MHT) on neurological and psychiatric disease risk have shown conflicting results across epidemiological and clinical studies. Given the widespread propagation of MHT among females experiencing menopausal symptoms, clarifying causality and impact is critical for informed decision-making. Although randomised controlled trials can establish causal effects, they are associated with significant costs and often of limited duration. Here, we present the first study using Mendelian randomisation (MR) to estimate causal effects of MHT on key neuropsychiatric and brain structural outcomes. To mimic the effects of MHT on oestrogen receptors (ER) α and β, we leveraged genetic variations located in the ESR1 and ESR2 genes that were significantly associated with positive controls as instrumental variables. In two-sample MR analyses, we used GWAS data on late-onset Alzheimer's disease (AD), key brain structural endophenotypes (cortical grey matter volume, hippocampal volume, and white matter hyperintensity volume), as well as depression and anxiety as outcomes, adjusting the p-values for multiple comparisons using false discovery rate correction with an α level of 0.05. There was no evidence for an impact of genetically proxied perturbation of ERα (β = -0.08, 95% CI [-0.34, 0.20], p=0.69 and β = -0.005, 95% CI [-1.45, 1.44], p=1.00) and ERβ (β = 0.35, 95% CI [-0.77, 1.47], p=0.69) on AD risk. Similarly, we found no significant impact of genetically proxied perturbation of ERα and ERβ on associated brain structural outcomes. Genetically proxied perturbation of ERβ was significantly related to higher depression risk (β = -0.66, 95% CI [-0.99, -0.32], p=0.002), but not anxiety risk. Our study provides support for a causal impact of genetically predicted oestrogen receptor perturbation on increased risk of depression, highlighting the importance of considering the implications of targeting these proteins with MHT, for psychiatric outcomes in clinical contexts. However, there was no support for either a harmful or protective causal effect of MHT on AD risk.

1. Introduction

Females are disproportionately affected by psychiatric and neurodegenerative disorders including depression, anxiety, and Alzheimer's disease (AD) [1, 2, 3]. One potential explanation for this female preponderance is an aetiological role of sex hormones such as oestrogens [4, 5, 6, 7]. Oestradiol, the most abundant and potent oestrogen in the female body, exerts dynamic effects on brain morphology, neurochemistry, and function [8, 9, 10, 11, 12], and is generally considered neuroprotective [13, 14, 15, 16]. During the menopause transition, menopausal hormone therapy (MHT) is often prescribed to alleviate symptoms [17, 18] by partially replenishing sex hormones [19, 20]. However, the effects of MHT use on mental health, AD risk, and markers of neurodegeneration are widely debated [4, 21, 22, 23, 24, 25], and the risks and benefits remain unclear.

MHT might help to mitigate neurological symptoms during the menopause transition [26, 27, 28, 29] with potential long-term implications for brain health [28, 30]. However, findings from observational studies on MHT use are mixed. Some studies report an increased risk of AD or dementia [31, 32, 33], and adverse effects on brain structure and neurodegenerative markers [34, 35, 36]. Other studies indicate a decreased AD risk [37, 38, 39, 40, 41] and beneficial effects on brain health [42, 43, 44], while some research finds no clinically relevant associations with neurological outcomes [45, 46, 47]. A meta-analysis of four randomised controlled trials (RCTs), based on data from the Women's Health Initiative Memory Study (WHIMS), found an increased risk of dementia with MHT use compared to placebo [30], though potential limitations of this dataset have been discussed [48]. The role of MHT in psychiatric disorders is also unclear. Some RCTs report a reduction in depressive symptoms with MHT use in menopausal females with depression [49, 50], as well as prevention of menopausal depression and anxiety [51, 52, 53]. Other RCTs reported no impact of MHT on depression and anxiety [27, 54, 55], while some observational studies have shown an increased risk of depression in MHT users [56, 57].

Discrepancies in findings across studies may partially arise from reverse causation or confounding factors, particularly in observational studies. For example, individuals with severe menopausal symptoms including cognitive difficulties or mood changes [58] may be more likely to be prescribed MHT [36, 45] or receive it following surgery (i.e., hysterectomy or

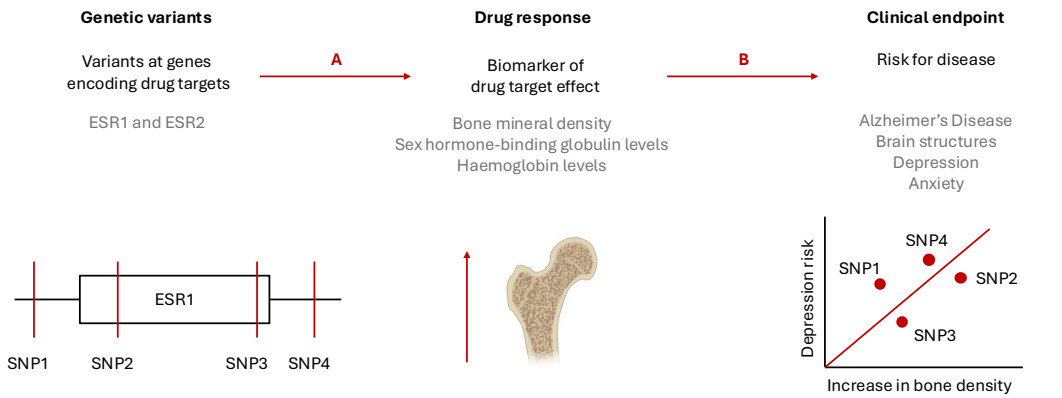
oophorectomy [59, 60]). Differences in factors such as MHT formulation, dosage, timing of onset, and duration of use are also likely to influence study results [4, 7, 30]. Furthermore, although RCTs are considered the gold standard in establishing causal effects [61], their limited duration may preclude examination of long-term outcomes like AD [30, 62]. Mendelian randomisation (MR) is an epidemiological method that utilises genetics in a quasi-experimental approach to estimate causal effects [63]. Random allocation of genetic variants predicting a given phenotype at conception is analogous to random allocation to intervention in RCTs [64]. Genetic variants cannot be influenced by environmental processes, which minimises concerns about confounding and reverse causation [63]. MR can be extended to investigate the causal effects of intervening on drug target proteins [65, 66, 67], including oestrogen receptor alpha (ERα) or oestrogen receptor beta (ERβ), which are targeted by oestrogen therapies such as MHT [13, 68, 69]. Genetic variants such as single-nucelotide polymorphisms (SNPs) that are located in the gene encoding the drug targets of interest (e.g., ESR1 and ESR2) can be used to proxy the effects of pharmacological intervention at a specific receptor if they are associated with downstream effects similar to the desired drug response [65, 70]. These SNPs can be used as instrumental variables in MR analyses to study the effect of these drug target perturbations on psychiatric and neurological outcomes [65]. With increasing availability of large-scale genetic data [71, 72], such drug-target MR studies present unprecedented opportunities to assess these relationships while avoiding resource constraints of RCTs and potential confounding in observational studies [73].

Here, we performed the first drug-target MR study examining the causal effects of genetically proxied ERα and ERβ perturbation on risk of AD, key brain structural outcomes, depression, and anxiety, given the female preponderance for these outcomes. To proxy drug target protein perturbation, we selected SNPs in the ESR1 and ESR2 gene that had previously been associated with relevant biomarkers representing downstream effects of drugs targeting these receptors. Specifically, these SNPs had been associated in genome-wide association studies (GWAS) with bone mineral density, SHBG levels, and haemoglobin levels at genome-wide significance ($p < 5 \times 10^{-8}$). We tested associations of these variants with AD, cortical grey matter (GM) volume, hippocampal volume, white matter hyperintensity (WMH) volume, depression, and anxiety.

2. Methods

2.1. Study design and instrument selection

Figure 1 illustrates the principles of MR studies used to investigate drug effects. By utilising genetic variants that proxy receptor activity, this approach can help us understand whether the binding of oestrogens to oestrogen receptors, the primary aim of MHT, has a direct impact on AD risk, brain structural outcomes, and mental health.



A) Instrument selection
Variants within ESR1 and ESR2 can instrument oestrogen receptor perturbation if they mimic its effects, e.g., increase bone mineral density.

B) Mendelian Randomisation
Effect of oestrogen receptor (ER) α and β perturbation on the study outcomes can now be tested using genetic instrumental variables.

Figure 1: Principles of Mendelian randomisation studies (MR) for studying drug effects. Genetic variants located within a gene encoding a drug target (e.g. in ESR1, which encodes oestrogen receptor α, one drug target protein of menopausal hormone therapy) that are associated with downstream biomarkers similar to the desired drug target effect (e.g. increased bone mineral density) are used as instrumental variables in MR analyses. Effects of genetically proxied perturbation on various outcomes (e.g. risk of depression) can be assessed. This figure represents an example and not the results from this study. SNP: single nucleotide polymorphism. Figure adapted from [65] with permission from the authors.

To proxy oestrogen receptor perturbation, we gathered all reported associations between SNPs located in the ESR1 and ESR2 genes and various biomarkers, based on the NHGRI-EBI GWAS Catalog (www.ebi.ac.uk/gwas/home) [74]. We selected only SNPs located within 200KB of the gene start and end (genome build 38p14; location for ESR1 = chromosome 6, 151,656,691 – 152,129,619, and for ESR2 = chromosome 14, 64,084,232 – 64,338,112) and associated with biomarkers at genome-wide significance ($p < 5 \times 10^{-8}$). We removed any associations observed in male-only samples to ensure we captured female-specific effects, or

non-European samples given that different genetic ancestries can influence allele frequencies and linkage disequilibrium (LD) patterns [75], and the lack of available ancestry-specific outcome GWAS. Table 1 provides an overview of exclusions for instrumental variable selection.

Biomarkers were chosen based on their biological plausibility to mimic drug target protein effects. For ESR1, we selected 'bone mineral density' and 'sex hormone-binding globulin (SHBG) levels'. Increased bone mineral density or reduced fraction risk has been observed in participants taking MHT compared to placebo in several RCTs [76, 77, 78, 79]. SHBG concentrations are significantly higher during use of hormonal contraception (which targets ERα) in a dose-response manner, as highlighted by a meta-analysis of experimental studies [80]. As there were two plausible biomarkers available, we selected both, as consistent results across these would strengthen our results. For ESR2, we chose the biomarker 'haemoglobin levels'. Females have lower haemoglobin levels compared to males [81], with times marked by increased levels of oestrogens (i.e., pregnancy) resulting in reductions in haemoglobin [82]. In transgender participants receiving oestradiol therapy, haemoglobin decreased significantly as oestradiol levels increased [83, 84].

Following biomarker selections, there were 30 SNP associations for bone mineral density, 5 for SHBG levels, and 4 for haemoglobin levels (Table 1); some SNPs were associated with a biomarker in several studies. Where SNPs associated with the same biomarker were in LD ($r2 < 0.1$), we selected a main SNP and its associated statistics based firstly on whether it had been identified in more than one sample, secondly the sample size of the study reporting the association, and thirdly the availability of summary statistics. Where the same SNP association was reported in several studies, summary statistics were obtained from the study with the largest sample size. All SNPs not in LD with any other SNPs were also selected as main SNPs. Summary statistics for the main SNPs were downloaded from the GWAS Catalog or retrieved manually from the publication. Supplementary Information (SI) Table 1, 2, and 3 provide the full list of studies reporting associations for SNPs in ESR1 and ESR2 with the biomarkers, including information on LD and availability of summary statistics. Table 2 displays the final selection of SNP associations comprising the three instruments reflecting ERα and ERβ perturbation.

Table 1: Overview of exclusions applied to the initial number of SNP associations reported for the ESR1 and ESR2 gene in the GWAS Catalog. SNP = single nucleotide polymorphism.

	ESR1	ESR2
Number of reported SNP associations	293	102
Number above $p < 5 \times 10^{-8}$	34	9
Number outside ± 200kb of gene region	0	0
Number of male-only samples	13	1
Number of non-European or mixed ancestry samples	97	22
Number of SNP associations following biomarker selections	35	4

Table 2: Main SNPs comprising the instruments reflecting oestrogen receptor α and β perturbation. All SNPs are intron variants. SNPs within each biomarker are uncorrelated. Summary statistics were obtained from Morris et al. (2019) [85], Kim et al. (2018) [86], Haas et al. (2020) [87], and Oskarsson et al. (2020) [88]. EA = effect allele, SE = standard error, SNPs = single nucleotide polymorphism, BMD = bone mineral density, SHBG = sex hormone-binding globulin, HMG = haemoglobin.

Gene	Biomarker	SNP	EA	Beta	SE	p-value	Study	Sample size
ESR1	BMD	rs2504069	C	-0.042	0.002	2.2×10^{-82}	Morris (2019)	426,824
		rs6905582	G	0.057	0.002	2.7×10^{-92}	Morris (2019)	426,824
		rs2982573	T	-0.077	0.002	1.1×10^{-305}	Morris (2019)	426,824
		rs2234693	T	-0.017	0.002	1.8×10^{-12}	Morris (2019)	426,824
		rs10484920	A	0.039	0.004	1.2×10^{-18}	Kim (2018)	394,929
		rs115192536	G	-0.053	0.004	6.4×10^{-35}	Kim (2018)	394,929
		rs547908752	C	0.08	0.007	4.0×10^{-27}	Kim (2018)	394,929
ESR1	SHBG	rs1738386	C	0.020	0.003	1.5×10^{-9}	Haas (2022)	196,901
ESR2	HMG	rs1256061	T	-0.021	0.002	1.6×10^{-23}	Oskarsson (2020)	684,122

2.2. Outcome selection

Our primary outcomes of interest were AD, depression, and anxiety, given the female preponderance of these conditions and the conflicting evidence surrounding associations with exogenous oestrogens [1, 4]. We further wanted to investigate associations with relevant brain structural outcomes; GM and hippocampal volume changes have been closely associated with the onset and progression of dementia [89], as well as depression [90, 91] and anxiety [92, 93]. Finally, as recent evidence highlights menopause as a risk factor for WMHs [94, 95], which have also been associated with increased risk of AD [96, 97], WMH volume was included as an outcome.

Variant-outcome associations were derived from large previously published GWAS. For AD, we selected a meta-analysis of GWAS on clinically diagnosed late-onset AD with 94,437 individuals [98]. Genetic associations with brain structural outcomes (cortical GM, hippocampal and WMH volume), were obtained from the largest GWAS of brain imaging phenotypes from the UK Biobank with 22,138 individuals [99]. For depression, we selected a meta-analysis of three GWAS on a spectrum of depression phenotypes with 807,553 individuals (246,363 cases and 561,190 controls) [100], and for anxiety, we chose a meta-analysis of nine GWAS on anxiety disorders with 17,310 individuals [101]. All genetic associations were based on GWAS of European ancestry samples.

2.3. Mendelian Randomisation

Two-sample MR analyses were used to obtain estimates for the association between genetically predicted oestrogen receptor perturbation, proxied by relevant biomarkers, and AD, cortical GM volume, hippocampal volume, WMH volume, depression, and anxiety. Analyses were conducted using the *TwoSampleMR* (version 0.5.6) package in R (version 4.2.1). Variants were harmonised between datasets, ensuring that the associations between SNPs and exposure and that between SNPs and the outcome reflected the same allele. For instruments with more than two variants, the Inverse Variance Weighted (IVW) [70] method was performed as the primary approach, which regresses the effect sizes of the variant-biomarker associations against the effect sizes of the variant-outcome associations. Several other methods, such as weighted median [102], weighted and simple mode [103], and MR Egger [104] were performed to assess robustness of results, as broadly consistent results across these methods strengthen the causal inference. For instruments with a single variant, the Wald ratio method was employed. To adjust for multiple testing, false discovery rate (FDR; 5%) [105] corrected p-values were calculated across all instances of IVW and Wald ratio methods. For any significant results, we conducted follow-up analyses that involved a standard MR analysis for the biomarker and the outcome, to assess whether the association was specific to the receptor perturbation proxies, or whether the biomarker itself had a significant causal association with the outcome (see SI section 2).

3. Results

3.1. Main findings

No significant associations were found between any instruments and AD or WMH volume (Figure 2, Tables 3, 4, and 5). Genetically predicted ERα perturbation, proxied by bone mineral density, showed associations with higher cortical GM volume (IVW β = 0.171, 95% CI [0.025, 0.317], p = 0.021), as well as lower hippocampal volume (IVW β = -0.192, 95% CI [-0.338, -0.046], p = 0.010), though neither association remained significant after FDR correction (p = 0.160 and p = 0.148, respectively) (Table 3).

Genetically predicted ERβ perturbation, proxied by haemoglobin levels, showed significant associations with higher risk of depression (Wald Ratio β = -0.656, 95% CI [-0.992, -0.319], $p < 0.001$, FDR-corrected p = 0.002) (Table 5, Figure 2). Genetically predicted ERα perturbation, proxied by bone mineral density, showed associations with higher anxiety (IVW β = 0.655, 95% CI [0.107, 0.319], p = 0.019), but this did not remain significant after FDR correction (p = 0.160) (Table 3).

4. Discussion

This MR study examined the effects of genetically predicted oestrogen receptor perturbation on neuropsychiatric outcomes and brain structure. In brief, we did not find significant effects on AD, brain structure, and anxiety, but genetically predicted ERβ perturbation was significantly associated with higher risk of depression.

4.1. Effects of perturbing MHT drug target proteins on AD and brain structure

There was no evidence for significant associations between genetically proxied perturbation of ERα or ERβ and AD, indicating that activity of these receptors, which are targeted by MHT, does not influence AD risk. This finding contrasts with WHIMS [106], which reported a doubled risk of developing dementia among postmenopausal women age 65 and older, though primarily for orally administered conjugated equine oestrogen therapy with progesterone [30]. Other trials enrolling younger females or administrating MHT closer to menopause have demonstrated mostly neutral effects of MHT on cognition [35, 52, 107, 108].

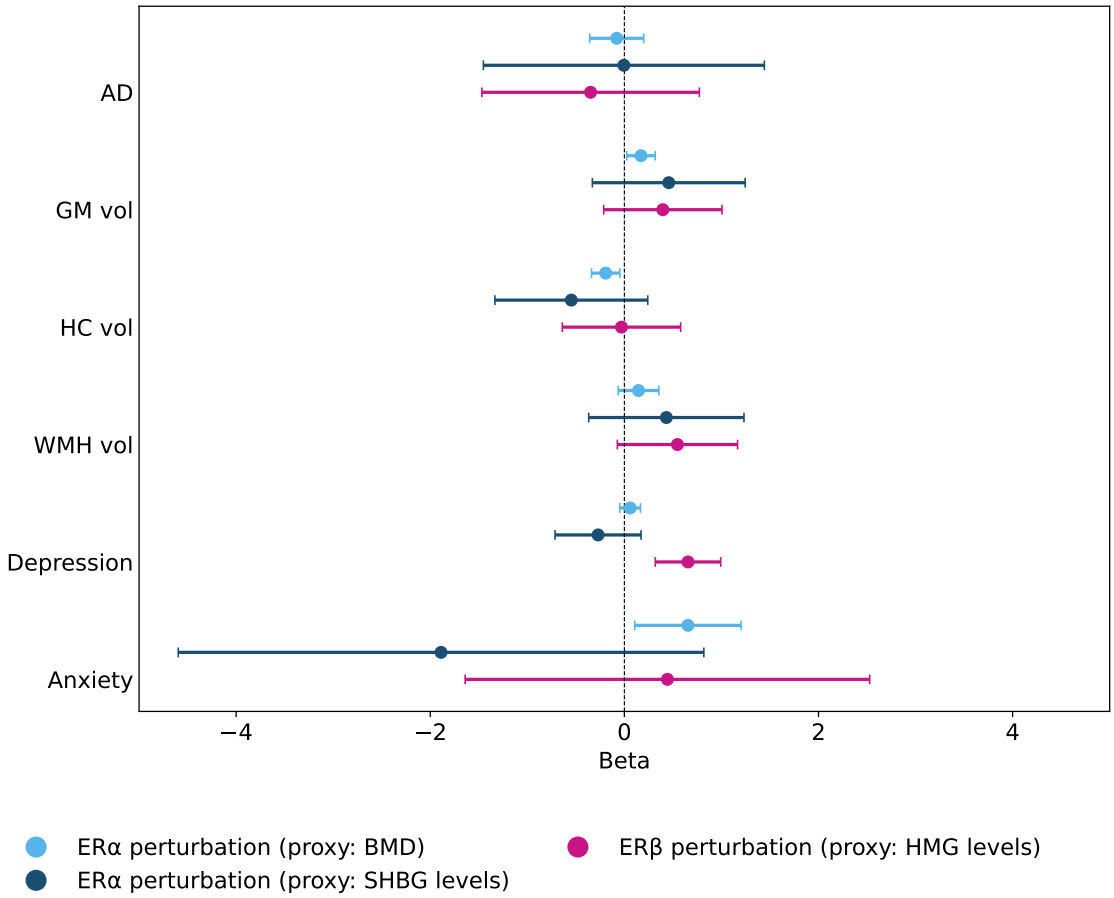


Figure 2: Forest plot showing two-sample MR estimates and 95% confidence intervals for the effects of ERα perturbation (proxied by bone mineral density (BMD) and sex hormone-binding globulin (SHBG) levels) and ERβ perturbation (proxied by haemoglobin (HMG) levels) on Alzheimer's Disease (AD), grey matter volume (GM vol), hippocampal volume (HC vol), white matter hyperintensity volume (WMH vol), depression, and anxiety. For associations between ERβ perturbation, all estimate signs have been switched for illustration purposes. Only estimates for inverse variance weighted (IVW) or Wald Ratio method are displayed. ER = oestrogen receptor.

Table 3: Two-sample MR estimates for ERα perturbation, proxied by bone mineral density, and all outcomes. Significant p-values (before or after FDR-correction) are marked in bold. IVW = inverse variance weighted; ER = oestrogen receptor; SNP = single nucleotide polymorphism; LCI = lower confidence interval; UCI = upper confidence interval; AD = Alzheimer's disease; GM = grey matter; HC = hippocampal; WMH = white matter hyperintensity.

Outcome	Method	SNPs	Estimate	LCI	UCI	p	p_{FDR}
AD	IVW	6	-0.079	-0.358	0.199	0.578	0.693
	Weighted median	6	-0.077	-0.400	0.245	0.638	
	Weighted mode	6	-0.077	-0.437	0.283	0.694	
	Simple mode	6	-0.128	-0.574	0.318	0.597	
	MR Egger	6	-0.021	-0.67	0.629	0.953	
Cortical GM volume	IVW	7	0.171	0.025	0.317	**0.021**	0.096
	Weighted median	7	0.163	-0.011	0.337	0.066	
	Weighted mode	7	0.176	-0.001	0.353	0.099	
	Simple mode	7	0.195	-0.068	0.458	0.196	
	MR Egger	7	0.112	-0.233	0.456	0.554	
HC volume	IVW	7	-0.192	-0.338	-0.046	**0.010**	0.089
	Weighted median	7	-0.165	-0.346	0.016	0.075	
	Weighted mode	7	-0.171	-0.35	0.008	0.109	
	Simple mode	7	-0.249	-0.546	0.047	0.150	
	MR Egger	7	-0.323	-0.667	0.022	0.126	
WMH volume	IVW	7	0.146	-0.064	0.356	0.172	0.391
	Weighted median	7	0.143	-0.029	0.315	0.104	
	Weighted mode	7	0.132	-0.055	0.318	0.216	
	Simple mode	7	0.196	-0.058	0.45	0.182	
	MR Egger	7	0.489	0.060	0.918	0.076	
Depression	IVW	6	0.060	-0.047	0.166	0.273	0.400
	Weighted median	6	0.055	-0.044	0.155	0.273	
	Weighted mode	6	0.047	-0.052	0.147	0.394	
	Simple mode	6	0.087	-0.086	0.259	0.371	
	MR Egger	6	0.106	-0.169	0.381	0.49	
Anxiety	IVW	6	0.655	0.107	1.203	**0.019**	0.096
	Weighted median	6	0.668	0.081	1.256	**0.026**	
	Weighted mode	6	0.686	0.008	1.364	0.104	
	Simple mode	6	0.251	-0.764	1.267	0.648	
	MR Egger	6	0.966	-0.300	2.233	0.209	

Table 4: Two-sample MR estimates for ERα perturbation, proxied by sex hormone-binding globulin levels, and all outcomes. Significant p-values (before or after FDR-correction) are marked in bold. IVW = inverse variance weighted; ER = oestrogen receptor; SNP = single nucleotide polymorphism; LCI = lower confidence interval; UCI = upper confidence interval; AD = Alzheimer's disease; GM = grey matter; HC = hippocampal; WMH = white matter hyperintensity.

Outcome	Method	SNPs	Estimate	LCI	UCI	p	p_{FDR}
AD	Wald ratio	1	-0.005	-1.452	1.442	0.995	0.995
Cortical GM volume	Wald ratio	1	0.457	-0.330	1.245	0.255	0.400
HC volume	Wald ratio	1	-0.547	-1.334	0.241	0.174	0.391
WMH volume	Wald ratio	1	0.433	-0.367	1.233	0.289	0.400
Depression	Wald ratio	1	-0.271	-0.714	0.172	0.230	0.400
Anxiety	Wald ratio	1	-1.889	-4.596	0.819	0.172	0.391

Table 5: Two-sample MR estimates for ERβ perturbation, proxied by haemoglobin levels, and all outcomes. Positive estimates indicate a negative relationship between ERβ perturbation and the outcomes due to perturbation being proxied by lower haemoglobin levels. Significant p-values (before or after FDR-correction) are marked in bold. IVW = inverse variance weighted; ER = oestrogen receptor; SNP = single nucleotide polymorphism; LCI = lower confidence interval; UCI = upper confidence interval; AD = Alzheimer's disease; GM = grey matter; HC = hippocampal; WMH = white matter hyperintensity.

Outcome	Method	SNPs	Estimate	LCI	UCI	p	p_{FDR}
AD	Wald ratio	1	0.348	-0.773	1.468	0.543	0.693
Cortical GM volume	Wald ratio	1	-0.396	-1.006	0.213	0.203	0.400
HC volume	Wald ratio	1	0.030	-0.580	0.640	0.924	0.978
WMH volume	Wald ratio	1	-0.547	-1.166	0.072	0.084	0.301
Depression	Wald ratio	1	-0.656	-0.992	-0.319	**<0.001**	**0.002**
Anxiety	Wald ratio	1	-0.444	-2.528	1.640	0.676	0.761

This discrepancy could potentially be explained by the critical window hypothesis, which suggests that the benefits of MHT are contingent on early initiation [109, 110, 111]. Although MR estimates can provide insights into lifelong effects of genetic variants, they do not equate to the impacts of pharmacological interventions initiated at specific times [65]. Therefore, despite the lack of evidence for a link between oestrogen receptor perturbation and AD, potential risks and benefits associated with timing of MHT initiation remain possible.

Furthermore, no evidence was found for associations between genetically predicted ERα or ERβ perturbation and brain structural outcomes, indicating that drugs targeting these receptors do not seem to impact cortical GM volume, hippocampal volume, or WMH volume. Although ERα was associated with higher cortical GM volume and lower hippocampal

volume, these findings did not remain significant after FDR correction. In a previous RCT study, recently postmenopausal females receiving conjugated equine oestrogens for four years had greater ventricular volume increases compared to placebo [35], but the increase was not different from placebo three years after discontinuation [108]. In a separate trial, there were no significant differences in total hippocampal volume between multiple doses of oestradiol and placebo following short-term administration, though higher bilateral posterior hippocampal volume was increased after three months at the highest dose [112]. Overall, the reported impact of MHT on GM changes ranges from volume increases, to decreases or null-effects (for a full review, see [6]), and comparability of studies is complicated by confounders such as age, mental health status, formulation and duration of MHT use [6, 7].

While relationships between MHT use and brain health might depend on formulation, dosage, timing of onset, and duration of use, the previously published GWAS, whose data we relied on, may also be subject to the healthy volunteer effect [113] and survivor bias [114]. These biases, which result from the tendency of healthier individuals to participate and the exclusion of those who have died or experienced severe illness, may affect the generalisability of our findings. Additionally, we primarily relied on variant-biomarker and variant-outcome associations reported in both males and females, mainly due to low availability of sex-stratified GWAS. Our results are therefore generalisable to both sexes (i.e., no associations between oestrogen receptor perturbation on AD risk in both males and females), but potentially underestimate sex-specific associations.

4.2. Effects of perturbing MHT drug target proteins on depression and anxiety

Genetically predicted ERβ perturbation, proxied by haemoglobin levels, was significantly associated with higher depression risk, supporting the role of oestrogens in mental health. Oestrogens are known to influence mood regulation through various mechanisms in the brain, such as modulating neurotransmitter systems and neuroplasticity [115, 116, 117], but the role of both endogenous and exogenous oestrogens in mood disorders is complicated and multifaceted. For instance, studies suggest that sensitivity to fluctuations in sex hormone levels may drive depressive symptoms in premenstrual dysphoric disorder (PMDD) or perimenopause [118, 119, 120]. Hormonal contraceptive use has been associated with increased risk of depression in observational studies [121, 122, 123, 124], especially in adolescents [123].

In contrast, a recent network meta-analysis including 14 RCTs concluded that hormonal contraceptive use did not lead to increased depressive symptoms in adult females [125]. Similarly, MHT use has been shown to be generally neutral [54, 55] or even protective [49, 50, 51, 52] for depressive symptoms in observational studies and RCTs.

Our study indicated an increased risk of depression associated with ERβ perturbation; however, as noted above, while our methods can provide evidence of the presence and direction of causal effects, they are not equivalent to pharmacological intervention [65]. Additionally, MR studies work under the assumption of constant genetic exposure effects over a lifetime, which may not capture the reality of time-varying effects [126]. As the relationship between oestradiol and psychiatric outcomes may change across different life stages [115], future MR studies could employ methods such as multivariable MR to examine time-varying effects [127], though this would require several GWAS on relevant traits across different age groups or timepoints in an individual's life.

We did not find evidence supporting a role of ERα in risk of depression. ERα perturbation was associated with higher anxiety, though this was not significant following FDR correction. Both receptor subtypes are located in brain regions associated with cognitive function and emotion [128, 129], but there is higher expression of ERβ in the thalamus and hippocampus [130], areas involved in mood disorders [131, 132, 133], which may explain our findings. Following future research confirming a differential role of ERα and ERβ in depression, oestrogen receptor modulators that selectively act on ERα could be explored to avoid inducing depressive symptoms with hormone therapy use.

There are some limitations in the use of haemoglobin as a proxy for ERβ perturbation. While higher oestrogen has been correlated with lower haemoglobin levels [81, 82, 83, 84], we could not identify any RCTs that linked use of exogenous oestrogens to lower haemoglobin levels. As haemoglobin level could not be clearly established as a downstream effect of MHT, its utility as a proxy may be less robust, for example in comparison to bone mineral density, the proxy for ERα perturbation. Additionally, as about two thirds of the participants in the haemoglobin level GWAS [88] were from the UK Biobank, we expected substantial sample overlap between the phenotype and outcome samples, which could introduce population-specific effects affecting the robustness of the results. Finally, standard MR follow-up analyses

showed a significant causal relationship between haemoglobin levels and depression (see SI section 2), indicating that our main results may not be specific to ERβ perturbation, though these results were not robust across sensitivity methods. Together, these considerations indicate that while haemoglobin shows promise as a proxy for ERβ perturbation, further research is needed to fully establish its reliability.

4.3. Clinical implications and future directions

This study represents an important initial step in using drug target MR studies to identify risks and benefits associated with MHT use. Although these studies cannot fully substitute for the precision and specific temporal dynamics captured in clinical pharmacological trials, this method can validate drug targets, identify relevant side-effects and outcomes, and aid in drug repurposing by identifying new therapeutic uses for existing drugs, without the high cost and long duration associated with RCTs [65, 66]. Triangulation of findings across these different methodological approaches is essential for a robust understanding of the causal effects of MHT.

Although our current results should be interpreted in the context of the discussed limitations, they provide a foundation for future research elucidating biological pathways and evaluating long-term safety and efficacy of MHT use to optimise women's healthcare oucomes. For example, well-powered sex-stratified GWAS are mostly lacking, and in addition to AD or psychiatric outcomes, GWAS on depression subtypes such as postpartum depression or PMDD would improve our understanding of female-specific findings related to hormonal treatments. Future analyses could also aim to examine the contribution of progesterone receptor perturbation on neuropsychiatric and brain structural outcomes. Exogenous oestradiol, via MHT or hormonal contraception, is commonly combined with progesterone to balance its effects [134], and various formulations might differentially affect risk of mental disorders [123, 135] as well as AD [24, 30, 136]. At the time of the current study, genetic variants within the PGR gene had not been associated with relevant biomarkers representing downstream drug effects, and we were thus unable to explore its role on these neuropsychiatric outcomes. Given the variety of MHT combinations, it is vital to conduct future studies that can provide a basis for clarifying causal effects of these on health outcomes.

5. Conclusion

This study found no evidence that genetically predicted ER perturbation, as targeted by MHT, significantly affects AD risk or associated brain structural measures including cortical GM volume, hippocampal volume, and WMH volume. However, ERβ perturbation may be associated with higher depression risk, indicating a potential causal role of oestrogen in mood regulation. Future drug target MR studies can complement observational studies and RCTs by offering critical insights into causal effects of MHT on various health outcomes. This approach is essential for clarifying inconsistent findings and guiding pharmaceutical research, ultimately optimising patient care and ensuring long-term health benefits for women.

Data availability statement

The data utilised in this study are available in the NHGRI-EBI Catalog of human genome-wide association studies or in the summary statistics from published GWAS.

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